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HOMOLOGY MODELING OF THE AMPA RECEPTOR: A QUANTITATIVE PREDICTIVE TOOL FOR THE DESIGN OF NOVEL ANTAGONISTS

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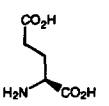
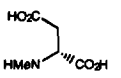
Abstract: A homology model of the AMPA receptor has been employed to construct a QSAR model of the AMPA receptor antagonist binding site. © 1997 Elsevier Science Ltd.

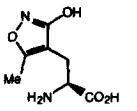
Excitatory neuronal transmission within the central nervous system (CNS) is mediated predominantly by ion flux through a family of cation selective ion channel complexes gated by the neurotransmitter L-glutamate **1**. Subclassification of these protein complexes, based upon the pharmacological properties of a series of unnatural agonists, has shown the family to be divided into three major subtypes: the *N*-methyl-(D)-aspartate (NMDA) **2** receptors (NR1 and NR2), the 2(*S*)-amino-3-(5′-methyl-3′-hydroxyisoxazoline)-propionic acid (AMPA) **3** receptors (GluR1-4) and the kainic acid (KA) **4** receptors (GluR5-7).[1] Compounds that inhibit AMPA receptors have been shown to be effective neuroprotectants under conditions of ischemic insult,[2] and in the treatment of epilepsy.[3] However, known AMPA antagonists do not exhibit ideal selectivity between AMPA and KA receptors. Consequently, we have been interested in the development of computational tools capable of directing the design and evaluation of novel selective AMPA receptor ligands.



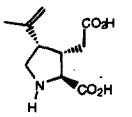
1 L-glutamate



2 NMDA



3 S-AMPA



4 Kainic Acid

Stern-Bach[4] suggested that the general topology of the agonist binding site of AMPA receptors is homologous to that of the bacterial periplasmic lysine/arginine/ornithine binding protein (LAOBP). Many examples of the bacterial periplasmic binding proteins have been analysed by X-ray crystallography both with and without associated ligands. A detailed study of chimeric GluR3/GluR6 receptors revealed a pattern of amino acid residues and sequences, which were shown to drive the AMPA/KA selectivity of these proteins. Construction of a simple homology model of this portion of the AMPA receptor, based upon the crystal structure of LAOBP, generated an initial bilobic model of the agonist binding domain. Mano[5] has elaborated upon this theme employing a slightly different alignment of the two proteins to generate a "Venus flytrap" model of the mechanism of action of the AMPA receptors. Sutcliffe[6] has recently proposed a different alignment of the related KA receptors with LAOBP. He has constructed the analogous homology model and has suggested a novel glutamate binding mode. Rationalization of this binding mode was based upon the known binding of orientation of lysine within LAOBP. Such a direct correlation is unproven. Paas[7] has analysed the related KBP proteins and has proposed a different model with which he has attempted to quantitatively rationalise agonist binding based upon the differences in observed binding to mutant receptor proteins. An extension of this work to antagonists is handicapped by the scarcity of data concerning antagonists in this study. Recently, we have published qualitative agonist binding site models of the NR1 and NR2B proteins based upon a series of mutagenesis studies.[8] Although there is no doubt that modeling of an unknown protein structure based upon the known coordinates of a

homologous protein is a valid method,[9] there is much disagreement as to how similar the two proteins should be, and how much detail should be extracted from the homology model. Comparative studies have indicated that the topographical differences between homologous regions of related proteins of low similarity can vary by as much as 6–10 Å. However, given that the known template is commonly used to generate an initial topology, which is subsequently modified during unconstrained minimizations, such studies should be regarded as potential sources of error rather than restrictive limitations. Comparison of the amino acid sequences of GluR3 and LAOBP show a very low level of both sequence similarity (28%) and identity (14%) within the identified regions. Thus, although the suggestion that these two proteins share a homologous topography is very appealing, it would be prudent to have some reservations concerning the accuracy and utility of such a correlation.

Predicting the affinity of ligands to protein structures has been an area of intense research over the past decade[10] with many studies focused on the precise prediction of binding energies. Less computationally intense approaches have shown the utility of simpler methods. A recent report concerning inhibitors of HIV proteases[11] demonstrated that while simple molecular mechanics calculations do not give realistic estimates of the binding energy, the calculated values may be readily correlated with the observed binding affinity. Here we provide details of a computational study of the interaction of a series of known competitive AMPA receptor antagonists **5–19** with an "atomic resolution homology model" of the agonist binding domain of GluR3, constructed from the crystallographic coordinates of unligated LAOBP.[12] As such our data supports to the idea that the agonist binding domain of the glutamate receptor proteins is topographically homologous to the bacterial binding proteins.

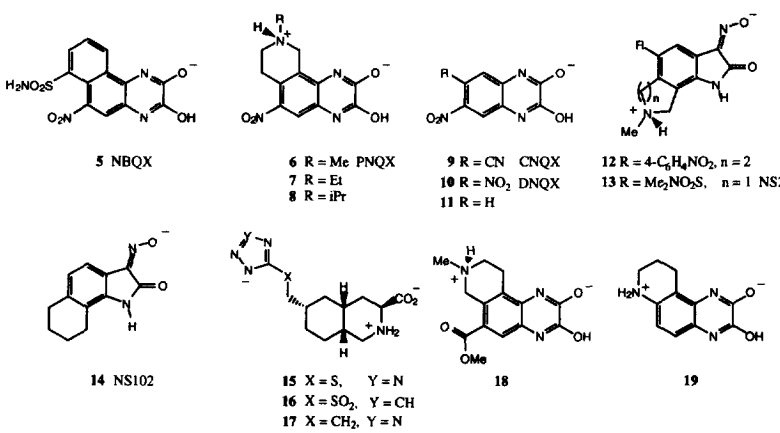


Figure 1. AMPA Receptor Antagonists

RESULTS

Employing the Stern-Bach[4] alignment of LAOBP and GluR3 as a starting point we have constructed a simple homology model of the agonist binding domain of the ion channel protein based upon the crystal structure of unligated LAOBP,[12] employing the protein modeling package of "Quanta v4.0" (Molecular Simulations Inc.). The regions hypothesized to adopt a secondary structure homologous to LAOBP were modeled by superimposing them on the corresponding regions of the template protein. Conversely, the sequences of GluR3 that were additional to the basic template structure were analysed by the standard algorithms for predicting protein secondary structure.[13] These sequences were modeled further by comparison to a protein structural library, and

then annealed onto the existing structural framework. In the absence of contradictory evidence the protein model employed the default ionization state for each residue. The model was minimized employing the CHARMm forcefield and all energy components. Minimization was performed initially holding the peptide backbone rigid (until the root mean square difference was <0.01), and then without constraints. Semi-systematic adjustment of the side chain conformations was perfomed by manual pairing of the charged residues, and repeated minimization.

Ligands **5–19**[14,15] were modeled in a fully ionized state. Individual ligands were placed within close proximity (2–3 Å) of the expected binding pocket, and the receptor/ligand complex was re-minimised. For each antagonist the conformation and orientation of the ligand within the binding pocket was systematically adjusted and the complex re-minimised. This process was repeated until all reasonable unique antagonist geometries had been explored. In most cases, reorientation of the ligand was found to occur resulting in highly similar structures. Only the lowest energy structure was retained after each iteration. The interaction energy ($E_{interact}$) for the individual ligands (Table 1) may be expressed as the difference between the calculated energy of the receptor/ligand complex and the energies of the individual receptor and ligand, eq 1. The volume of the ligands was computed employing a 0.5 Å grid overlaying the van der Waals surface of the final bound conformation of the individual species. Rotatable bonds for a particular ligand were assessed as those bonds whose position in the bound complex were necessarily fixed by interactions with the protein surface. Regression of this data with the observed affinity of these ligands gave the relationship in eq 2 ($r^2 = 0.752$, cross-validated $r^2 = 0.482$,).

$$E_{interact} = E_{receptor+ligand} - (E_{receptor} + E_{ligand}) \qquad \text{eq 1}$$

$$\text{Affinity} = -0.0157E_{interact} + 0.0137Vol_{ligand} - 1.211Rot_{bonds} + 1.723 \qquad \text{eq 2}$$

Ligand	IC_{50} (^{3}[H]AMPA) (μM)	$p(IC_{50})$	$E_{interact}$	Volume (Å^3)	Rot. Bonds	Predicted $p(IC_{50})$	Residual
5	0.052	7.28	-232	234	1	7.36	-0.08
6	0.080	7.10	-266	216	2	6.44	+0.66
7	0.166	6.78	-271	245	2	6.91	-0.13
8	0.153	6.82	-263	256	2	6.94	-0.12
9	0.34	6.47	-205	173	1	6.11	+0.36
10	0.20	6.70	-216	170	1	6.24	+0.46
11	2.00	5.70	-220	151	1	6.04	-0.34
12	0.15	6.82	-175	290	1	7.23	-0.41
13	0.70	6.15	-155	250	1	6.37	-0.22
14	7.20	5.14	-145	201	1	5.55	-0.41
15	0.58	6.24	-183	240	2	5.46	+0.78
16	0.63	6.20	-195	257	2	5.87	+0.33
17	1.35	5.87	-190	238	2	5.55	+0.32
18	44.0	4.36	-285	248	4	4.75	-0.39
19	24.0	4.62	-225	179	2	5.28	-0.66

Table 1. Measured and Computed Affinities of AMPA Receptor Antagonists

DISCUSSION

Many approximations and simplifications are inherent in the use of protein homology models for ligand design: however, the wealth and breadth of data presently available from mutagenesis studies and other protein characterization techniques presents the modeler with a rich source of comparative check points to evaluate the accuracy of any proposed homology model. Mutagenesis studies of the AMPA receptor have indicated a series of residues "involved" in the binding of both agonists and antagonists to the AMPA binding site,[5,16] notable

residues are E425, D470, K472, and R508. All mutations of R508, including R508K completely abolished functional activity of the protein complex. All mutations of the other 3 residues lead to varying reductions in the efficacy of both agonists and antagonists. Inspection of our ligand/protein model complexes reinforces these results (Figure 2). The topography of the pocket may best be described as a blind canyon between the two lobes of the model. While E425, D470 and K472 constitute a major portion of one of the canyon walls, residues from the upper lobe and the interlobe crossover sequences constitutes the other. A flap, which possibly holds the bound ligand in the pocket, is formed by the side chain of R508. As such the amino acid residues contributing to this gross topology ensure that the walls of the canyon are heavily charged. These features appear to be similar to the KBP model proposed by Paas.[7] Thus, the receptor surface may interact strongly with either charged species such as the endogenous ligand glutamate, or the polycyclic aromatic antagonists typified by NBQX. Comparison of the position of each of the bound ligands within the lowest energy complexes suggested that these 15 antagonists all interact with the receptor surface in a similar manner. However, closer examination reveals that there are actually two classes of ligands within those examined. The polycyclic aromatic species, **5–14** and **18–19**, appear to interact predominantly with the walls and the bottom of the canyon, whilst the perhydroisoquinolines **13–15** preferentially interact with the walls and the lower surface of lobe II.

Figure 2. Stereo View of NBQX **5** in the Homology Model of the GluR3 Binding Pocket

To quantify the interaction of these ligands with the receptor model we have constructed a series of QSAR equations correlating various parameters with the observed affinities of these ligands. A simple plot of the measured interaction energy against the observed affinity of these 15 ligands gave a poor statistical correlation ($r^2 = 0.432$). This is not surprising since it is well understood that the observed affinity of a ligand for a particular receptor is governed by more than just the interaction enthalpy for the ligand/protein complex.

The individual components of the overall interaction energy would be expected to include both enthalpy and entropy terms relating to both the formation of the ligand/protein complex and the desolvation of the two components. Without a substantially more complex computational approach the enthalpy of desolvation would be difficult to quantify. As such, we assumed that for the ligands concerned in this study the cost of desolvation is essentially constant. However, the entropic terms may not necessarily be constant. Whilst the entropic cost of binding nonflexible ligands such as the majority of those employed in this study might be expected to be small, a major component of the overall stability of the complex is due to the entropy gained as a result of displaced water molecules returning to the bulk solvent medium. The displacement of water molecules must be a step function of the ligand size and shape, however a rough correlation of the entropy gain due to solvent displacement can be

attained by considering the overall volume of the ligands. Whilst the correlation of $E_{interact}$ and Vol_{ligand} with the observed affinity was better than purely $E_{interact}$, a number of outliers were observed. These ligands are in fact the more flexible species. The entropic costs associated with binding such ligands have been estimated to be proportional to the number of rotatable bonds within the ligand. Regression of the observed affinity against the $E_{interact}$, Vol_{ligand} and Rot_{bonds}[17] gave the correlation in eq 2. Such a degree of correlation is comparable to other studies employing structural templates derived directly from protein crystallographic data.

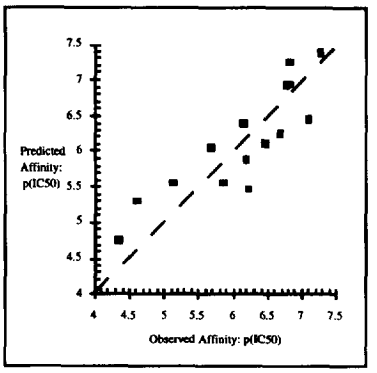


Figure 3. Plot of Predicted Affinity vs. Observed Affinity Employing Eq 2 (r^2 = 0.752, CV r^2 = 0.482, BS r^2 = 0.756, F-test = 11.103, N_{var} = 4, N_{obs} = 15, DF = 11).

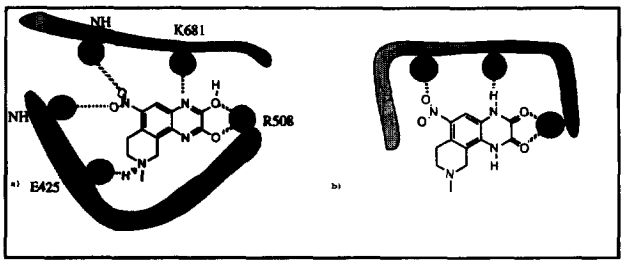


Figure 4. Comparison of 2 Models of the AMPA Binding Pocket: (a) LAOBP Generated Homology Model of the GluR3 Binding Pocket and (b) QSAR Derived AMPA Pharmacophore.[14]

One of the common biases of simple QSAR models involves the relative alignment of the individual ligands. Consequently most structure-activity studies have focused upon one class of ligands, which makes complete mapping of the binding pocket unlikely. A study of NBQX-like ligands at the AMPA receptor was recently reported (Figure 4b),[14] from which an antagonist pharmacophore was subsequently developed. This model was employed to rationalize the reported data, however the study did not extend to the perhydroisoquinoline antagonists **15–17**. Comparison of the homology model surface, as bound by ligands **5–19**, with the published AMPA receptor pharmacophore shows a good degree of overlap. Both models predict that the binding of antagonists is associated with a strong electrostatic interaction at one end of the binding pocket, and a series of spacially defined hydrogen bonding interactions along the bottom/top of the receptor pocket. The electrostatic interaction of the quinoxalinedione ring is generated through an interaction with R508 (Figure 4a and 4b). In agreement with the earlier study we also find a weak hydrogen bonding interaction with the C_8 substituent of the quinoxalinedione unit. A major difference is that the published study employed neutral ligands, while we explored the interaction of appropriately charged ligands. We feel that such species are more compatible with both the nature of the ligands and the surface features of the receptor model. Indeed, an earlier working version of our model employing uncharged ligands gave a poor correlation with the observed affinity of these ligands. Thus, the interaction of the ligands with R508 is most likely a hydrogen bridged ionic interaction. Additionally, unlike the 3-D QSAR study, we find a similar hydrogen bridged charge/charge interaction involving the non-aromatic ring nitrogen atom of species such as PNQX, **6**. At physiological pH it is reasonable to assume that such a basic nitrogen should be in the protonated form. Accordingly E425 appears to be involved in a strong interaction with this array, rationalising the high affinity of this template (**6–8**, **12**, and **13**).

A number of assumptions were made regarding the nature of the interactions of these ligands with the receptor, but not the alignment of the ligands within the pocket. Therefore, it is very interesting to observe that the perhydroisoquinoline antagonists appear to interact to a greater extent with the upper surface of the receptor pocket (lower surface of lobe II) than do the other ligands. Consequently, it is easy to understand why such species were not included in the aforementioned study, since the electron rich tail of these species extends into a region not generally occupied by the other ligands.

Although the data shown does not lead to a perfect correlation between the receptor model/ligand complexes and the observed affinity of the ligands, the correlation supports both the protein homology model of GluR3, and the interactions apparent between ligand and receptor. Indeed, while a typical QSAR is useful for "interpolation" of affinity data, it does not allow for "extrapolation" beyond the scope of the original ligands. In the absence of crystal structures, homology models such as presented and the associated quantitative correlations allow for extrapolation and design of novel drug candidates.

In conclusion, employing the concept of homology modeling, we have generated a predictive model of the AMPA receptor. This model lends support to the hypothesis that the topological architecture of the agonist binding domain of GluR3 and LAOBP are similar. Additionally our results elaborate upon a recently proposed AMPA receptor pharmacophore. We believe that this work demonstrates that useful binding site models may be generated through protein homology modeling even in the case of low similarity proteins.

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